108 Adaptive mechanisms associated with increased virulence and persistence of *Burkholderia cenocepacia* during chronic lung infection: A quantitative proteomic analysis

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Burkholderia cepacia complex (Bcc) infections in cystic fibrosis (CF) are associated with a worse prognosis and increased risk of death. Understanding the mechanisms involved in bacterial virulence and in genetic adaptation of these bacteria to the CF lung is crucial for an improved management of Bcc chronic infections [1]. A 2 decade-long systematic survey of Bcc respiratory infections in the major Portuguese CF Centre has allowed us to carry out a retrospective study of the adaptive clonal variation of these pathogens within the airways during lung function deterioration [1–4]. In this work, we assessed the virulence of 3 *B. cenocepacia* clonal isolates spanning a 3.5-year long chronic infection in a single CF patient, based on their ability to invade epithelial cells and open tight junctions. The isolates retrieved later before the patient's death with cepacia syndrome were found to be significantly more virulent than the one that initiated the infection. A genome-wide quantitative proteomics approach was exploited to identify differentially expressed proteins and infer mechanisms that could be associated with *B. cenocepacia* virulence and persistence in the lung (ref. [2], unpublished results). Overall, the results are suggestive of adaptation to microaerophilic conditions and iron availability, and highlight the important role of metabolic optimization in adaptation and increased pathogenic potential of *B. cenocepacia* during the course of a chronic CF infection.

Reference(s)

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109 The *Burkholderia cepacia* small colony variants (SCV) are a more pathogenic bacterial form that may facilitate persistent respiratory infections in CF patients

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Pulmonary colonization of cystic fibrosis (CF) patients with bacteria of the *Burkholderia cepacia* complex (Bcc) is associated with worse prognosis and increased risk of death. In CF respiratory infections, *B. cenocepacia* and *B. multivorans* are the most commonly species isolated worldwide, but a remarkable exception was registered at the major Portuguese CF Center at Hospital de Santa Maria (HSM), in Lisbon, with an exceptionally high presence of *B. cepacia* [1,2]. The present study is focused on two pairs of small and normal colony clonal variants of *B. cepacia* isolated from a CF patient at the beginning and at a late stage of infection. This is the first report on the presence of both *B. cepacia* small colony variants (SCV) and normal colony morphotype clonal bacteria in the CF airways, since respiratory infections with this species are sporadic. Relevant phenotypes were compared and SCV were found more resistant to all the antimicrobials tested and to U.V. radiation. Compared to the normal morphotypes, SCV do have a higher capacity for biofilm formation and exhibit a lower degree of saturation of membrane lipids, as reported for normal *B. cenocepacia* clonal isolates retrieved during the late stages of chronic infection [3]. The SCV are more virulent than the corresponding normal forms, as suggested by the use of *C. elegans* infection model. These results point to SCV as pathogenic forms of *B. cepacia* that may facilitate persistent and recurrent respiratory infections in CF patients.

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110 Quantitative exoproteomic analysis to better understand the mechanisms underlying *Burkholderia cenocepacia* persistence and virulence in CF lung infections

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Pulmonary colonization with *Burkholderia cepacia* complex (Bcc) bacteria in cystic fibrosis patients is largely associated with a worse prognosis and increased risk of death. These bacteria adapt to the stressing lung environment, immune defences and antibiotic therapy and are virtually impossible to eradicate from the lung. The whole set of proteins secreted by bacteria (exoproteome) during host–pathogen interaction plays a crucial role in lung infection, since these proteins participate in adhesion to host cells, invasion, damage of host tissues, resistance to several stresses, and disruption of the host's immune system. Therefore, extracellular proteins are potential targets for the development of diagnostics, vaccines and antimicrobial therapies. We have applied a quantitative exoproteomic approach to elucidate the adaptive mechanisms employed by *B. cenocepacia* during chronic colonization of the CF lung. The exoproteomes of 3 sequential clinical isolates, retrieved from the same patient from the onset of infection until death with cepacia syndrome [1] and grown under oxygen limitation, were compared. Proteins with different abundance in the 3 clinical isolates include several virulence factors, such as the extracellular zinc metalloprotease ZmpA, type IV and VI secretion systems proteins, proteins involved in the alteration of LPS structure and peptidoglycan biosynthesis, and the virulence factor AidA. Other extracellular and membrane-associated metabolic proteins were also identified, suggesting a paramount role of metabolic adaptation in bacterial persistence in the lungs.

Reference(s)

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111 The *Burkholderia cenocepacia* J2315 small non-coding RNA MavA is required for fitness and virulence

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Bacteria of the *Burkholderia cepacia* complex (Bcc) are opportunistic pathogens that can cause deadly lung infections, particularly among cystic fibrosis patients [1]. A major problem associated with Bcc infections is their intrinsic resistance to the most clinically-relevant antibiotics, rendering their eradication very difficult [2]. Therefore, the identification and characterization of new virulence determinants and mechanisms in these bacteria is of critical importance for the identification of novel targets that can be used in the design of new strategies to battle Bcc infections.
In this work we describe the isolation and characterization of a mutant strain derived from *B. cenocepacia* J2315, carrying a mutation in the *mavA* sRNA. This sRNA is 108 nucleotides long and its nucleotide sequence was found as highly conserved within the genomes of the Bcc strains sequenced so far.
The *mavA* mutant exhibited significant cell surface alterations, increased biofilm formation and reduced virulence in the nematode *Caenorhabditis elegans*. The mutant also exhibited an increased susceptibility to the b-lactams imipenem and ceftazidime, to tetracycline, and to sodium dodecyl sulphate. Altogether, our findings suggest that MavA is required for the bacterial survival to stressful environments and full virulence. Experimental and bioinformatics results showing that MavA affects important cellular processes in *B. cenocepacia* will be also presented.

Reference(s)

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